HA stem. Whittle et al. (2014, J Virol, 88(8): p. 4047-57) describe H1 HA comprising a Y98F mutation in the amino acid sequence of H1 that inhibits SA binding while permitting host-cell binding. Since native HA proteins bind to SA on B cells and cause a high level of background 'noise' in studies that focus on binding between the B cell receptor and its cognate antigen, Whittle describes the use of the Y98F-HA as a probe to detect HA-specific B cell receptor interaction in patients that have previously been vaccinated with an H5 influenza virus.

[0014] WO2015183969 describes nanoparticle-based vaccine consisting of a novel HA stabilized stem (SS) without the variable immunodominant head region genetically fused to the surface of nanoparticles (Gen6 HA-SS np, also referred to as Hl-SS-np). WO2015183969 found that Hl-SS-np induced effective signaling through wild-type B cell receptor. However, nanoparticle with full-length HA containing Y98F mutation to abolish nonspecific binding to sialic acid (HA-np), induced wild-type B cell receptor to a lesser extent, suggesting a reduced immune response to HA with Y98F mutation.

[0015] The receptor binding site is located on the globular head of HA and amino acid 98 is at the base of the receptor binding site. The phenol side chain of Y98 forms a hydrogen bond with sialic acid to facilitate binding. Phenylalanine has a similar structure to tyrosine so that the shape of the binding pocket and antigenicity is maintained by the Y98F mutation. However, phenylalanine lacks a hydroxyl group on the side chain and therefore cannot form hydrogen bonds with sialic acid. While the Y98F substitution prevents HA binding to SA, the overall structure and conformation of HA remains intact (Zost S. J., et. al., 2019, Cell Rep. 29:4460-4470).

[0016] The potential role of cognate and non-cognate interactions between HA and host cells on influenza vaccine outcomes using suprastructures, for example, protein complexes, or VLPs comprising a modified HA that reduces binding of the modified HA to sialic acid (SA) is described herein.

SUMMARY ~~OF THE INVENTION~~

[0017] The present ~~invention~~disclosure relates to suprastructures or virus like particles (VLPs) that comprise modified influenza hemagglutinin (HA) protein. The modified HA protein comprises one or more than one alteration that reduces interaction of the modified HA to sialic acid (SA), the interaction might be a non-cognate interaction.

[0018] According to the present ~~invention~~disclosure there is provided a suprastructure comprising modified influenza hemagglutinin (HA), the modified HA comprising one or more than one alteration that reduces non-cognate interaction of the modified HA to sialic acid (SA) of a target, while maintaining cognate interaction, with the target. Furthermore, it is provided a suprastructure comprising modified influenza hemagglutinin (HA), the modified HA comprising one or more than one alteration that reduces non-cognate interaction of the modified HA to sialic acid (SA) of a protein on the surface of a cell, while maintaining cognate interaction with the cell.

[0019] For example, the modified HA may comprise one or more than one alteration that reduces binding of the modified HA to sialic acid (SA), while maintaining cognate interactions, with a target or a cell. Non-limiting examples of the target may include a B cell receptor, and/or one or more targets comprising a B cell surface receptor that comprises SA. Non-limiting examples of a cell may include B cell and non-limiting examples of protein on the surface of the cell may include B cell surface receptor.

[0020] The alteration that reduces binding of the modified HA to SA may comprise a substitution, deletion or insertion of one or more amino acids within the modified HA. Furthermore, the suprastructure may be a virus like particle (VLP). A composition comprising the suprastructure or VLP, and a pharmaceutically acceptable carrier, a vaccine comprising the composition, and a vaccine comprising the composition in combination with an adjuvant are also described.

[0021] Also provided herein is a plant or portion of a plant comprising a suprastructure or VLP comprising modified influenza hemagglutinin (HA), the modified HA comprising one or more than one alteration that reduces binding of the modified HA to sialic acid (SA) to a target or protein on the surface of a cell, while maintaining cognate interactions with the target or cell. Non-limiting examples of the target may include a B cell receptor, and/or one or more targets comprising a B cell

animal or subject, of a second vaccine comprising a composition comprising virus like particles comprising a corresponding wild type HA.

[0037] As described herein, use of a modified HA protein, a suprastructure (protein suprastructure), or VLP comprising the modified HA protein, as an influenza vaccine was observed to increase immunogenicity and efficacy when compared to the immunogenicity and efficacy of an influenza vaccine comprising a corresponding parent HA that does not comprise the modification that results in reduced, non-detectable, or no non-cognate interaction with SA, for example, reduced, non-detectable, or no SA binding. The parent HA that does not comprise the modification that results in reduced, non-detectable, or no non-cognate interaction with SA may include a non-modified HA, a wild type influenza HA, an HA comprising a sequence that is altered, but the alteration is not associated with SA binding, a suprastructure or VLP comprising the parent HA, a wild type influenza HA, or the HA comprising a sequence that is altered, but the alteration is not associated with SA binding.

[0038] This summary of the ~~invention~~disclosure does not necessarily describe all features of the ~~invention~~disclosure.

[0038a] In one aspect, the present disclosure refers to a virus like particle (VLP) comprising modified influenza hemagglutinin (HA), the modified HA comprising one or more than one alteration that reduces non-cognate interaction of the modified HA to sialic acid (SA) of a protein on the surface of a cell, while maintaining cognate interaction with the cell.

[0038b] In another aspect, the present disclosure refers to use of the VLP as disclosed herein in the manufacture of a medicament for modulating an immunological response, or for increasing an immunological response.

[0038c] In another aspect, the present disclosure refers to a composition comprising the VLP as disclosed herein and a pharmaceutically acceptable carrier.

[0038d] In another aspect, the present disclosure refers to a composition comprising a mixture of more than one VLP as disclosed herein and a pharmaceutically acceptable carrier, wherein one or more than one VLP comprises one type of modified HA or wherein one or more than one VLP comprises a mixture of modified HA or wherein one or more than one VLP comprises one type of modified HA and one or more than one VLP comprises a mixture of modified HA.

[0038e] In another aspect, the present disclosure refers to a vaccine comprising the composition as disclosed herein.

[0038f] In another aspect, the present disclosure refers to a plant or portion of a plant comprising the VLP as disclosed herein.

[0038g] In another aspect, the present disclosure refers to a modified influenza hemagglutinin (HA) comprising one or more than one alteration that reduces binding of the modified HA to sialic acid (SA) of a protein on the surface of a cell, while maintaining cognate interaction with the cell.

[0038h] In another aspect, the present disclosure refers to a virus like particle (VLP) comprising the modified HA as disclosed herein.

[0038i] In another aspect, the present disclosure refers to a nucleic acid encoding the modified HA as disclosed herein.

[0038j] In another aspect, the present disclosure refers to a plant or portion of a plant comprising the nucleic acid as disclosed herein.

[0038k] In another aspect, the present disclosure refers to use of the vaccine as disclosed herein or the composition as disclosed herein in the manufacture of a medicament for inducing immunity to influenza virus infection in an animal or subject in need thereof.

[0038l] In another aspect, the present disclosure refers to use of a first vaccine comprising the vaccine as disclosed herein in the manufacture of a medicament for increasing an immunological response in a first animal or a subject in response to an antigen challenge, wherein the immunological response is increased when compared

with a second immunological response in a second animal or subject that has been administered a second vaccine comprising virus like particles comprising a corresponding unmodified HA.

[0038m] In another aspect, the present disclosure refers to a method of producing a virus like particle (VLP) comprising, expressing the nucleic acid as disclosed herein within a host under conditions that result in the expression of the nucleic acid and production of the VLP.

[0038n] In another aspect, the present disclosure refers to a method of producing a virus like particle (VLP) comprising modified HA in a plant or portion of a plant comprising, introducing the nucleic acid as disclosed herein within the plant or portion of the plant, and growing the plant or portion of the plant under conditions that result in the expression of the nucleic acid and production of the VLP.

[0038o] In another aspect, the present disclosure refers to a method of producing a virus like particle (VLP) comprising modified HA in a plant or portion of a plant comprising, growing a plant, or portion of a plant that comprises the nucleic acid as defined as disclosed herein, under conditions that result in the expression of the nucleic acid and production of the VLP.

[0038p] In another aspect, the present disclosure refers to a vaccine comprising the modified HA as disclosed herein and a pharmaceutically acceptable carrier.

[0038q] In another aspect, the present disclosure refers to use of the vaccine as disclosed herein in the manufacture of a medicament for increasing an immunological response of an animal or a subject in response to an antigen challenge, wherein the immunological response is increased when compared with an immunological response in a second animal or subject that has been administered a vaccine comprising an influenza HA that does not comprise the one or more than one alteration.

[0038r] In another aspect, the present disclosure refers to a suprastructure comprising modified influenza hemagglutinin (HA), the modified HA comprising one or more than one alteration that reduces non-cognate interaction of the modified HA to sialic

acid (SA) of a protein on the surface of a cell, while maintaining cognate interaction with the cell.

[0038s] In another aspect, the present disclosure refers to use of the suprastructure as disclosed herein in the manufacture of a medicament for modulating an immunological response, or for increasing an immunological response.

BRIEF DESCRIPTION OF THE DRAWINGS

[0039] These and other features of the invention will become more apparent from the following description in which reference is made to the appended drawings wherein:

[0040] **FIGURE 1A** shows a sequence alignment of the amino acid sequences of hemagglutinin (HA) of A/California/7/09 (H1N1) (SEQ ID NO:2); A/Idaho/7/18 (H1N1) (SEQ ID NO:101); A/Brisbane/02/18 (H1N1) (SEQ ID NO: 195); A/Kansas/14/17 (H3N2) (SEQ ID NO: 61); A/Minnesota/41/19 (H3N2) (SEQ ID NO: 13). A/Indonesia/5/2005 (H5N1) (SEQ ID NO: 14); A/Egypt/NO4915/14 (H5N1) (SEQ ID NO:108; A/Shanghai/2/2013 (H7N9) (SEQ ID NO: 21); A/Hangzhou/1/13 (H7N9) (SEQ ID NO: 109); Outlined residues align with amino acids Y98 of HA from influenza H3 strains, for example A/Kansas/14/17 (H3N2) (SEQ ID NO: 61). Signal peptides have been removed for clarity. **FIGURE 1B** shows a sequence alignment of the amino acid sequences of hemagglutinin (HA) of B/Phuket/3703/13 (Yamagata lineage) (SEQ ID NO:28); B/Singapore/INFKK-16-0569/16 (Yamagata

WHAT IS CLAIMED IS:

1. A virus like particle (VLP) comprising modified influenza hemagglutinin (HA), the modified HA comprising one or more than one alteration that reduces non-cognate interaction of the modified HA to sialic acid (SA) of a protein on the surface of a cell, while maintaining cognate interaction with the cell.

2. The VLP of claim 1, wherein, the modified HA is being selected from:

i) a modified H1 HA, wherein the one or more than one alteration is Y91F; wherein the numbering of the alteration corresponds to the position of reference sequence with SEQ ID NO: 203;

ii) a modified H3 HA, wherein the one or more than one alteration is selected from Y98F, S136D; Y98F, S136N; Y98F, S137N; Y98F, D190G; Y98F, D190K; Y98F, R222W; Y98F, S228N; Y98F, S228Q; S136D; S136N; D190K; S228N; and S228Q; wherein the numbering of the alteration corresponds to position of reference sequence with SEQ ID NO: 204;

iii) a modified H5 HA, wherein the one or more than one alteration is Y91F; wherein the numbering of the alteration corresponds to position of reference sequence with SEQ ID NO: 205;

iv) a modified H7 HA, wherein the one or more than one alteration is Y88F; wherein the numbering of the alteration corresponds to position of reference sequence with SEQ ID NO: 206;

v) a modified B HA, wherein the one or more than one alteration is selected from S140A; S142A; G138A; L203A; D195G; and L203W; wherein the numbering of the alteration corresponds to position of reference sequence with SEQ ID NO: 207; or

vi) a combination thereof.

3. The VLP of claims 1 or 2, wherein the modified HA is selected from:

i) a modified H1 HA, wherein the one or more than one alteration is Y91F; wherein the numbering of the alteration corresponds to the position of reference sequence with SEQ ID NO: 203;

ii) a modified H3 HA, wherein the one or more than one alteration is S136D; wherein the numbering of the alteration corresponds to position of reference sequence with SEQ ID NO: 204 or

iii) a modified B HA, wherein the one or more than one alteration is D195G; wherein the numbering of the alteration corresponds to position of reference sequence with SEQ ID NO: 207, or

iv) a combination thereof.

4. The VLP of any one of claims 1 to 3 wherein the cell is a B cell.

5. The VLP of any one of claims 1 to 4, wherein the protein on the surface of the cell is a B cell surface receptor.

6. Use of ~~The~~ the VLP of any one of claims 1 to 5 in the manufacture of a medicament for~~for use in~~ modulating an immunological response, or for increasing an immunological response.

7. The ~~VLP~~ use of claim 6, wherein the immunological response is a cellular immunological response, a humoral immunological response, or both a cellular immunological response and a humoral immunological response.

8. A composition comprising the VLP of any one of claims 1-~~7~~ 5 and a pharmaceutically acceptable carrier.

9. A composition comprising a mixture of more than one VLP as defined in any one of claims 1 to ~~8~~ 5 and a pharmaceutically acceptable carrier, wherein one or more than one VLP comprises one type of modified HA or wherein one or more than one VLP comprises a mixture of modified HA or wherein one or more than one VLP comprises one type of modified HA and one or more than one VLP comprises a mixture of modified HA.

10. The composition of claim 9, wherein the one or more than one VLP comprises one type of modified HA.

11. A vaccine comprising the composition of any one of claims 8-10.

12. The vaccine of claim 11, wherein the vaccine further comprises an adjuvant.

13. A plant or portion of a plant comprising the VLP of any one of claims 1-~~7~~5.

14. A modified influenza hemagglutinin (HA) comprising one or more than one alteration that reduces binding of the modified HA to sialic acid (SA) of a protein on the surface of a cell, while maintaining cognate interaction with the cell.

15. The modified HA of claim 14, wherein the modified HA is being selected from:

i) a modified H3 HA, wherein the one or more than one alteration is selected from Y98F, S136D; Y98F, S136N; Y98F, S137N; Y98F, D190G; Y98F, D190K; Y98F, R222W; Y98F, S228N; Y98F, S228Q; S136D; S136N; D190K; S228N; and S228Q; wherein the numbering of the alteration corresponds to position of reference sequence with SEQ ID NO: 204;

ii) a modified H5 HA, wherein the one or more than one alteration is Y91F; wherein the numbering of the alteration corresponds to position of reference sequence with SEQ ID NO: 205;

iii) a modified H7 HA, wherein the one or more than one alteration is Y88F; wherein the numbering of the alteration corresponds to position of reference sequence with SEQ ID NO: 206;

iv) a modified B HA, wherein the one or more than one alteration is selected from S140A; S142A; G138A; L203A; D195G; and L203W; wherein the numbering of the alteration corresponds to position of reference sequence with SEQ ID NO: 207; or

v) a combination thereof.

16. The modified HA of claim 15, wherein the modified HA is further selected from: a modified H1 HA, wherein the one or more than one alteration is Y91F; wherein the numbering of the alteration corresponds to the position of reference sequence with SEQ ID NO: 203.

17. The modified influenza HA of any one of claims 14-16, wherein the cell is a B cell.

18. The modified influenza HA of any one of claims 14-16, wherein the protein on the surface of the cell is a B cell surface receptor.

19. The modified HA of any one of claims 14-16, wherein the modified HA comprises plant-specific N-glycans or modified N-glycans.

20. A virus like particle (VLP) comprising the modified HA of any one of claims 14-16.

21. The VLP of claim 20 further comprising one or more than one lipid derived from a plant.

22. A nucleic acid encoding the modified HA of any one of claims 14-16.

23. A plant or portion of a plant comprising the nucleic acid of claim 22.

24. ~~A method of inducing immunity to influenza virus infection in an animal or subject in need thereof, comprising administering~~ Use of the vaccine ~~as defined in~~of claim~~s~~ 11 or 12 or the composition of any one of claims 8-10 in the manufacture of a medicament for inducing immunity to influenza virus infection ~~to~~in ~~the~~ an animal or subject in need thereof.

25. The ~~method~~ use of claim 24, wherein the vaccine is to be administered to the animal or the subject orally, intradermally, intranasally, intramuscularly, intraperitoneally, intravenously, or subcutaneously.

~~26. A use of the vaccine of claims 12 or 13 or the composition of any one of claims 8-10 for inducing immunity to influenza virus infection in an animal or subject in need thereof.~~

~~27~~26. Use of a first vaccine comprising the vaccine of claim 11 or 12 in the manufacture of a medicament for~~A method of~~ increasing an immunological response in a first animal or a subject in response to an antigen challenge~~ comprising~~, ~~administering a first vaccine, the first vaccine comprising the vaccine of claims 12 or 13 to the animal or subject and determining the immunological response, and~~ wherein the immunological response is increased when compared with a second immunological response in a second animal or subject~~, obtained following administration of~~ that has been administered a second vaccine comprising virus like particles comprising a corresponding unmodified HA.

~~28~~27. A method of producing a virus like particle (VLP) comprising, expressing the nucleic acid of claim 22 within a host under conditions that result in the expression of the nucleic acid and production of the VLP.

~~29~~28. The method of claim 28, wherein the host is harvested and the VLP is purified.

~~30~~29. A method of producing a virus like particle (VLP) comprising modified HA in a plant or portion of a plant comprising, introducing the nucleic acid of claim 22 within the plant or portion of the plant, and growing the plant or portion of the plant under conditions that result in the expression of the nucleic acid and production of the VLP.

~~31~~30. A method of producing a virus like particle (VLP) comprising modified HA in a plant or portion of a plant comprising, growing a plant, or portion of a plant that comprises the nucleic acid as defined in claim 22, under conditions that result in the expression of the nucleic acid and production of the VLP.

~~32~~31. The method of claims ~~30~~ 29 or ~~31~~30, wherein the plant or portion of the plant is harvested and the VLP is purified.

~~33~~32. A vaccine comprising the modified HA of any one of claims 14-16 and a pharmaceutically acceptable carrier.

~~34~~33. Use of the vaccine of claim 32 in the manufacture of a medicament for~~A method of~~ increasing an immunological response of an animal or a subject in response to an antigen challenge~~ comprising, administering the vaccine of claim 33 to the animal or subject and determining the immunological response~~, ~~and~~ wherein the immunological response is increased when compared with an immunological response in a second animal or subject ~~obtained following administration of~~that has been administered a vaccine comprising an influenza HA that does not comprise the one or more than one alteration.

~~35~~34. The ~~method~~ use of claim ~~27~~ 26 or ~~34~~33~~.~~ , wherein the immunological response is a cellular immunological response, a humoral immunological response, or both a cellular immunological response and a humoral immunological response.

~~36~~35. A suprastructure comprising modified influenza hemagglutinin (HA), the modified HA comprising one or more than one alteration that reduces non-cognate interaction of the modified HA to sialic acid (SA) of a protein on the surface of a cell, while maintaining cognate interaction with the cell.

~~37~~36. Use of ~~The~~ the suprastructure of claim ~~36~~35 in the manufacture of a medicament~~,~~ for ~~use in~~ modulating an immunological response, or for increasing an immunological response.